ŒSTRUS AND FECUNDITY IN THE GUINEA PIG

DONALD B. TRESIDDER

DEPARTMENT OF ANATOMY, STANFORD MEDICAL SCHOOL

THIS study was undertaken at the suggestion of Professor Meyer, primarily for determining the numerical relation between the corpora lutea of pregnancy and implantations in the guinea pig.

Most of the animals used in this experiment were purchased from dealers, for it was impossible, in the short time at my disposal, to obtain young animals of uniform age and with the exception of a few guinea pigs raised in our laboratory, only approximate ages were known.

The guinea pigs were housed in a well-lighted, sunny, heated room. Lantz, '13, reported that the optimum temperature for the guinea pig is 65°. Draper, '20, stated that they thrive best at temperatures between 50° and 70° and found young animals extremely susceptible to small changes in temperature; some of them dying when the temperature was lowered permanently from 60° to 58° F. However, I did not notice any marked difference in the behavior or condition of extremely young animals kept at a temperature of 50°. They showed every sign of vigor and no animals were lost as a result of this exposure. Indeed, I learned of guinea pigs kept in the open in unheated pens, sheltered only from wind and rain. These animals were said to thrive and to multiply at the customary rate, but no records were kept. In my own work I found that a few degrees above or below 50° seemed to make no appreciable difference in the behavior of the animals, and I hence am somewhat sceptical about the marked susceptibility of the guinea pig to cold, so often reported.

The animals were fed dry alfalfa and barley daily and green vegetables about twice a week. Many writers have reported that guinea pigs did not do well on dry feed, but it was my experience that, if fed an abundance of water, they throve on alfalfa and barley alone. Since they are subject to intestinal disturbances, it is of con-

siderable importance that they be fed with the greatest regularity.

Several animals were lost during the course of the experiments and in each case a necropsy was performed. Illness, in several of the animals, extended over a period of weeks. They lost steadily in weight, and tended to assume a characteristically crouching attitude. The fur became rough and tousled. Some of them chewed incessantly, although some pain seemed to be associated with the process. The full significance of this behavior was made clear at the necropsy. Guinea pig No. 7, for example, which succumbed after an illness of three weeks, had an empty stomach, and the abdominal cavity was absolutely devoid of fat. There were no macroscopic signs of infection or disease. Examination of the teeth revealed that the upper incisors were worn down almost to the gums, with a more than corresponding increase in the length of the lower incisors, making occlusion of the molars impossible. The molars were loose and could easily be picked from the jaw with an ordinary laboratory forceps.

The body of guinea pig No. 12, which died with practically the same symptoms, showed extreme atrophy and emaciation. Ascaroid parasites were found in the rectum. The upper incisors were loose and worn and the short stumps remaining could be be removed with the fingers. The upper and lower incisors were separated by about 8 mm., due to the fact that the molars occluded first and prevented the short, probably fractured incisors from meeting. From the findings in these cases it would seem that guinea pigs may die of starvation because of the presence of worn or irregular teeth and consequent inability to masticate food. It may perhaps be that the changes in the teeth of these animals were due to senility, but further observations are necessary to confirm this before a definite answer can be given to the question.

In order to study daily stages in the pregnancy of guinea pigs it became necessary to mate a large number of animals and to know the exact time of copulation. Stockard and Papanicolaou, '17, studied the œstrous rhythm of

the guinea pig by making microscopic examination of the material found in the vagina. They found that "Guinea pigs kept in a state of domestication and under steady environmental conditions possess a regular dicestrous cycle, repeating itself in non-pregnant females about every sixteen days throughout the entire year, with probably small and insignificant variations during the different seasons. Each period of sexual activity lasts about 24 hours and is characterized by the presence of a definite vaginal fluid which is not sufficiently abundant to be readily detected on the vulva, but is easily observed by an examination of the interior of the vagina." They added that macroscopic signs of heat are unreliable.

In my work it was found impractical to determine the existence of heat microscopically and the knowledge that heat should recur about every fifteen days furnished a starting point. Each female was given a number and entered on an individual record sheet giving the following data:

Date and hour of attempted mating.
Result of attempted mating.
Each time the animal came into heat the record showed:
Whether heat was recognizable by macroscopic examination.
Number of days since last heat.
Number of hours since the first successful coitus.
Number of hours that external signs of heat could be observed by examination.

Matings were attempted daily, whether the animal was supposed to be pregnant or not. The males were introduced into the pens with the females regardless of whether or not the latter were thought to be in heat, and they were allowed to remain with the females from five to fifteen minutes. It was easy to follow the dicestrous cycle of any individual animal. A glance at the guinea pig's record each day showed the number of days since the last heat, and, knowing that heat should return about the fifteenth day, it was practically impossible for it to come and go unnoticed unless it recurred altogether irregularly. We

found that after some practise heat could be determined rather accurately by inspection. A guinea pig in rut will often assume the position of copulation when stroked gently over the lumbar region. The vulva are swollen and moist, and often a cheesy secretion is seen. The latter is a positive sign of heat, but we found that some guinea pigs refused to mate during the entire period in which the secretion was present.

In young animals we found heat recurring every fifteen or sixteen days with very little variation among individuals of the same age. Three striking exceptions in which heat returned in twelve days will be reported later in this paper. Papanicolaou and Stockard found that in old multiparæ the period may be lengthened to 18 days. I also found that as the animals grow older they seem to become more and more irregular in their rhythm. In three very old animals I was unable to find any signs of heat throughout an entire year, although I attempted to mate them twice daily. Three other animals maintained a cycle of 20 days, and in some cases we were unable to demonstrate any regular œstrous rhythm at all, either by inspection or by the use of a male.

Subsequently (1920) these workers have reported that "underfeeding with a diet of 20 grams of carrots per day produces prolongation of the dicestrum, and at the same time a congestion in the ovary and uterus and a degeneration of developing Graafian follicles." They concluded that "the extent of prolongation of the dicestrum depends upon the stage at which an animal is underfed. . . . Large follicles seem to require better nutrition than a small primary follicle. . . . Thus a late underfeeding has a more injurious effect than an early one, and postponement of the next œstrus is correlated with a postponement of new ripe follicles in the ovary." Stockard and Papanicolaou believe that the ovarian follicles are extremely sensitive to environmental conditions. They believe that extreme variations in the œstrus cycle of certain animals may be accounted for, partially at least, by differences in nutrition.

In the course of these observations the intervals be-

tween attempted matings were shortened, with the idea that heat might be recurring unnoticed, but mating never occurred at other intervals. It is doubtful whether any definite rhythm is maintained by old guinea pigs, for pig No. 9, which was observed to be in heat December 27, was not in heat again until 49 days later. Animal No. 20 was in heat October 17 and heat did not return until 91 days later. In another instance heat returned after 118 days. However, since the age of these animals is not known, it is impossible to be sure that these irregularities are due to senility.

Bischoff, '44, stated that copulation in the guinea pig occurs within 3 hours after parturition. In four cases in which he prevented copulation heat returned after intervals of 40, 50, 51, and 51 days. Hensen, '76, and Rein, '83, claimed that the most favorable time for copulation is within one hour after parturition. I observed copulation in 12 animals immediately after parturition. Matings were attempted at one-hour intervals for six hours afterward. In four cases I was unable to mate the females at this time. They were found in heat again 34, 36, 81, and 120 days later. The first two animals were about six months old. The last two were very old, judging by their teeth. Two females mated 1 hour after parturition, 2 after 2 hours, 1 after 3 hours, 1 after 5 hours, and 2 after 6 hours. In three cases no pregnancy resulted and heat returned in 31, 31 and 29 days.

Many writers have reported that females refuse the male shortly after the first copulation. The inference is that some nervous mechanism automatically terminates heat soon after copulation. Instances have been reported in which the female refused the male 20 minutes after the first copulation. In observations extending over nearly a year, however, three cases were observed in which the female mated again eight hours after the first copulation. In the majority of cases the female permitted copulation three hours after the first mating. One animal mated 13 times in an interval of 8 hours. It seems that a female accepts the male at any time during the first stage of heat regardless of any previous intercourse, but apparently

she permits matings somewhat reluctantly after this. Instead of assuming the position for copulation when approached by the male she often runs around the cage and resists vigorously. Unless the male is very persistent and active copulation will not occur. One female resisted a second coitus for fifteen minutes by kicking, snapping, etc., only to stop suddenly and take the position for copulation. This behavior of the female may be due to previous mating or it may simply mean that the period of heat is subsiding. I am inclined to the latter view, because we have encountered many females among animals which had not been previously mated, who resisted the males vigorously for a time, only to yield in the end. The time during which the females permitted copulation unhesitatingly was a relatively short one, but after this phase had passed the animal might yet be mated if the male was persistent.

Stockard and Papanicolaou, '17, are of the opinion that among domesticated guinea pigs only a slight seasonal variation exists in the occurrence of heat, but in the present series of guinea pigs the fall months were the most favorable for matings, as shown by the following table:

Month	Number of Matings	Resulting Pregnancies	Percentage
September	23	21	91.3%
October	17	10	58.8
November	8	3	37.5
December	11	4	36.3
January	7	4	57.1
February	5	0	0.0
March	8	4	50.0
April	12	5	41.6
May	9	5	55.5
June	6	5	83.3

The males seemed to be partly responsible for this wide variation. During the winter months they were lethargic and indifferent. When placed in a pen with a female known to be in heat, the male often ignored her, eating unconcernedly instead. In many instances several males had to be placed with such a pig, in succession, before a mating took place. This is in marked contrast to the customary behavior, for when placed in a pen with two

females, the male will often go directly to the female in rut. Sometimes, however, he will mistakenly pursue the one that is not in heat, although repelled by sharp bites and other negations, only to wheel suddenly and mount the receptive female. The pursuit of the wrong animal may only serve to stimulate him, but in some instances it was necessary to remove her before he would turn his attentions to the one in rut. Puzzling sexual idiosyncrasies also were noted. Instances were observed, for example, in which a male would not under any circumstances mate with a certain female which was in heat, although he was persistent in the case of others. On the other hand, some females also were noticed to repulse a certain male, although accepting others.

It will be seen from Table I that 106 matings resulted in 61 pregnancies, or 57.5 per cent. Draper, '20, reported that only 40 per cent. of the animals bred by him became pregnant. Since Stockard and Papanicolaou found 95.4 per cent. out of 88 pigs pregnant, considerable variation would seem to exist. The large discrepancy between their results and ours may be due to the fact that the latter were working with uniformly young, selected animals or that the males were left to remain with the females, instead of being removed after several copulations.

In the many matings not followed by pregnancies, the next œstrous cycle was prolonged. This is shown by the accompanying chart.

Guinea-pig Number	Heat returned after
21	30 days.
32	44
22	28
27	15
50	46
52	29
8	30
9	31
39	12
37	15
3	29
5	30
8	15
16	30

As noticeable in the above chart, the lengthened diœstrous periods are nearly exact multiples of 15, the normal period, thus showing that the cycle is definitely periodic as reported by Stockard and Papanicolaou, '17*b*. Long, '15, found that the œstrous cycle was prolonged by inserting a glass rod in the vagina of the rat. He held this prolongation to be due to a stimulation of the cervix of the uterus. Although I stimulated the uterus of guinea pigs by means of a warm glass rod in three cases only, heat returned in 15, 15 and 16 days, and I regret that I was not able to extend this series of experiments in order to obtain more data on this interesting phenomenon revealed by Long in the rat. However, from the above table, it is clear that copulation definitely prolongs the next œstrous cycle in the majority of cases. This may be due to direct stimulation of the cervix of the uterus, as explained by Long, or implantation may have occurred, followed by abortion or by absorption of the young conceptuses, in cases in which the period was greatly prolonged.

Guinea pig No. 39 (see Table II) was mated two hours after parturition, but no pregnancy followed. This animal was remated 12 days later, with resulting pregnancy. This confirms a case reported by Rubasckhin, '05, in which heat returned 10 days after parturition. Stockard and Papanicolaou, in considering Rubasckhin's report, regarded 10 or 12 days as too short a period to indicate the return of heat. Nevertheless, in the case reported here heat was unmistakable, and this animal which was mated 12 days after parturition became pregnant. I observed heat to return in 12 days also in two other pigs.

Young animals constantly in association with males became pregnant at an earlier age than females isolated from males. Of a litter containing 3 females and 1 male, two females were placed in separate cages a few days after birth and the remaining male and female were allowed to run together. At the age of 5 months, the latter produced a litter. This indicates that the mating of this pair occurred before the animals were three months old. Yet no ill effects of this early mating or of the inbreeding could be detected in the offspring.

When the two sisters were two months old, males were introduced into the pens twice daily, but no signs of heat were observed, and no matings occurred until these females were five months old. Similar results were obtained with two other litters. Since my work was done Mr. Warnock, a fellow student, has observed two females to bear viable litters at the end of the third month. This implies mating at the early age of one month. The paternal male was several months older, however.

The Corpora Lutea of Pregnancy

In order to study the correlation between corpora lutea and implantations during the various stages of pregnancy, animals were mated and killed, from the seventh day of gestation on, for each day up to and including the fifteenth. From the fifteenth day to full term, animals were killed every other day.

When the guinea pigs were killed, the ovaries and uteri were removed and placed in formalin for twenty-four hours and the number of embryos in each horn of the uterus recorded. The ovary corresponding to the horn of the uterus having the larger number of conceptuses was arbitrarily chosen for use in determining what relation might exist between the number of conceptuses and the number of corpora lutea. Thus guinea pig No. 10 had two conceptuses in the right horn and one in the left. The right ovary was embedded and cut serially into thick sections. The left ovary was cut 7 micra thick for the study of changes in the corpora lutea during pregnancy.

In a study of 14 embryos, Draper, '20, found 76 in the left horn and 69 in the right, a ratio of 1 to 0.9. Of 98 embryos from 35 guinea pigs, I found 55 in the right horn and 43 in the left, a ratio of 1 to 0.78. The average number of fœtuses per pregnancy was three.

Table II shows that there is a marked agreement between the number of corpora lutea in an ovary and the number of implantations in the corresponding horn of the uterus. Out of 34 ovaries examined, the number of corpora lutea was the same as the number of embryos

in the corresponding horn of the uterus in all save six cases. In five of these six instances there was one embryo less in the horn of the uterus than there were corpora lutea in the ovary. In the other case, the right

TABLE II

Guinea Pig	Duration of Pregnancy	Embryos		Corpora Lutea		Remarks
		Right	Left	Right	Left	
35	7	1	1	3	1	Well-formed C.L. but no external evidence of implantations.
34	8	0	3	1	3	
33	9	2	1	2	1	
32	10	1	3	1	3	
31	11	1	1	1	3	Well-marked evidence of resorption.
30	12	3	0	3	0	
29	13	1	2	1	2	
28	14	3	1	3	1	
27	15	0	1	0	2	
26	17	3	0	2	0	
25	19	1	3	1	3	
24	21	2	1	2	1	
23	23	2	3	2	3	
22	25	3	0	3	0	
21a	27	3	0	2	0	
20	29	3	1	3	1	
19	31	2	1	2	1	
18	33	2	0	3	1	
17	35	2	1	2	0	
16	37	3	1	3	1	
15	39	2	1	2		
14	41	1	2	1	2	
13	43	1	2	1	2	
12	45	1	2	2	2	Conceptus on left side almost completely resorbed.
11	47	1	1	2	1	
10	49	2	1	2	1	
9	51	2	1	2	1	
8	53	1	2	1	2	
7	55	2	0	2	0	
6	57	1	1	2	1	
5	59	1	2	2	2	
4	61	2	1	2		
3	63	3	1	3		
2	Term	2	1	2	1	
A	23	0	0	2	0 (A)	C.L. of pregnancy but no implantations found.
B	45	0	0	1	1 (B)	

horn showed 3 embryos although only two corpora lutea of pregnancy were present in the ovary. Hence, in this case, two embryos developed from a single ovum or a single follicle contained two ova. In the instances where there was one more corpus luteum than embryos it is possible that another conceptus was present and became

absorbed or that an ovum degenerated before implantation, or that it failed of fertilization.

As shown by Meyer, '17 and '19, and Stockard and Papanicolaou, '18, absorption is not uncommon in the uteri in guinea pigs. In this series, three embryos which were clear-cut cases of absorption were found upon examination of the uteri after their removal. In No. 12, which was killed forty-five days after copulation, two normal embryos were found in the left horn, but in the right horn there was nothing but a small mass which had undergone almost complete absorption.

According to Stockard and Papanicolaou, '18, embryos eight or ten days old may be detected by "carefully feeling the uterus through the body wall of the mother." They report a case as follows:

> A normally developed embryo 19 mm. crown rump length is shown in Fig. 6 and near it is seen an amorphous embryonic mass 2 mm. in longest diameter which represents the other member of the litter. . . . The entire mass of the smaller ovum in the uterus was about that of a ten-day specimen, while the normal individual was a typical 20-day specimen. This case was detected by external examination and was merely opened in order to use the embryos for illustrating the phenomenon.

Although I used the method of Papanicolaou and Stockard in palpating guinea pigs, in no instance was I able to determine the number of embryos with certainty under fifteen days. Because of this fact, I found it necessary to sacrifice the animals in order to determine the number of implantations before this period.

Guinea pig No. 35 and guinea pig No. 34 were killed seven and eight days after conception, respectively, and the uteri removed. Careful palpation of the removed uteri failed to reveal the number of conceptuses. The uteri were then opened, but in order to determine the number of implantations present it was necessary to embed them and make serial sections. From this I am led to question the possibility of determining the number of embryos in the uterus by palpation through the abdominal wall on the eighth to tenth day of pregnancy. This skepticism seems warranted, further, by the measurements of

three ten-day conceptuses, *6.5 × 3 mm.*; *6.8 × 4.5 mm.*; *6.5 × 4.5 mm.* respectively. Draper gave the estimated length of an 11-day *embryo* measured under magnification as 2 mm.

Stockard and Papanicolaou (1918) likewise reported that a "slightly cystic ovary" has frequently been diagnosed by palpation through the abdominal wall of the guinea pig. In my observations 23 out of 75 ovaries were found to be cystic; but the largest cyst measured only *1.6 mm.* × *1.68 mm.* and not even this could by any chance have been palpated through the abdominal wall. Hence, it would seem that Stockard and Papanicolaou must have been dealing with markedly large and unusual, rather than with slightly, cystic ovaries.

From a study of a large series of gestations in the domestic pig, Corner, '21, concluded that internal migration of ova is relatively common. This small series of pregnancies in the guinea pig furnishes very little evidence upon this question, for such a possibility is suggested only by No. 17, a pregnancy of 35 days in which there were 2 corpora and 2 implantations on the right side and no corpora but one implantation on the left side. Since the total number of implantations in this case exceeds that of corpora, one must assume that one ovum divided or that one follicle contained ova and that one of the ova arising from the right then migrated to the left cornu. However, since this pregnancy was so far advanced, this assumption implies that a corpus luteum of pregnancy in the guinea pig can not be wholly resorbed in 35 days and that it never fails to form.

It is of special interest in this connection that a second case of this kind has been observed in this laboratory by Miss Clark. In this case there were two corpora in the left ovary and none in the right, with one implantation on each side. Since this pregnancy was only 17 days old, the question of early resorption of the corpus luteum probably can be excluded with considerable certainty but that of failure of the corpus luteum to form, remains.

REFERENCES

Bischoff, H. L. W.
1844. Beweis der von der Begattung unabhängigen periodischen Reifung und Loslösung der Eier der Sängethiere und des Menchen. Entwicklungsgeschichte des Meerschweinchens.

Corner, George N.
1921. Internal Migration of the Ovum. *J. H. H. B.*, Vol. 32.

Draper, R. L.
1920. The Prenatal Growth of the Guinea Pig, *Anat. Rec.*, Vol. 18, No. 4, May, 1920.

Evans, H. M. and Long, J. A.
1920. The Œstrus Cycle in the Rat (et seq.). *Anat. Rec.*, Vol. 18.

Lantz, David.
——. The Raising of the Guinea Pig. U. S. Dept. of Agriculture, Farmers' Bulletin, 525.

Long, J. A.
1919. The Œstrus Cycle in Rats. *Proc. Am. Soc. Zool., Anat. Rec.*, Vol. 15, 1919.

Meyer, A. W.
1917. Intrauterine Absorption of Ova. *Anat. Rec.*, Vol. 12, 1917.
1919. Uterine, Tubal and Ovarian Lysis and Resorption of Conceptuses. *Biol. Bull.*, Vol. 36, April, 1919.

Rein,
1883. Beitrage zur Kenntnis der Reifungsercheinungen und Befruchtungsvorgange am Saugetierei, Arch. f. Mikr. Anat., vol. 22.

Rubaschkin, W.
1905. Ober die Reifungs- und Befruchtungs-processe des Meerschweincheneies. *Anat. Hfte.*, Abt. 1, Vol. 29, 1905.

Stockard, C. R. and Papanicolaou, G. N.
1917*a*. The Existence of a Typical Œstrus Cycle in the Guinea Pig, —with a Study of Its Histological and Physiological Changes. *Am. Jour. Anat.*, Vol. 22, No. 2, Sept., 1917(*a*).
1917*b*. A Rhythmical "Heat Period" in the Guinea Pig. *Science*, N. S., Vol. 46, pp. 42, 44, 1917(*b*).

Stockard, C. R. and Papanicolaou, G. N.
1918. Further Studies on the Modification of the Germ-cells in Mammals: the Effect of Alcohol on Treated Guinea Pigs and their Descendants. *Jour. Exp. Zool.*, Vol. XXVI, p. 119, May, 1918.
1919. The Vaginal Closure Membrane, Copulation and the Vaginal Plug in the Guinea Pig, with Further Considerations of the Œstrus Rhythm, *Biol. Bull.*, Vol. XXXVII, Oct., 1919.

Stockard, C. R. and Papanicolaou, G. N.
1920. Effect of Underfeeding on Ovulation and the Œstrous Rhythm in Guinea Pigs. *Proc. Soc. Exper. Biology and Medicine*, XVII, 1920.

Hensen, V.
1876. Beobachtungen über die Befruchtung und Entwickelung des Kaninchens und Meerschweinchens. *Zeit. f. Anat. u. Entwick.*, Bd. I, 1876.